

Deciphering small molecules as new virulence factors in the bacterial sugarcane pathogen *Xanthomonas albilineans*

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Xanthomonas albilineans is a xylem-invading plant pathogen that causes leaf scald disease of sugarcane. Unlike most plant pathogenic bacteria, *X. albilineans* does not possess a Hrp-Type Three Secretion System. Pathogenicity of this bacterial species must therefore rely on other virulence factors. *X. albilineans* produces albicidin, a toxin and potent inhibitor of DNA gyrase which inhibits prokaryotic DNA replication. Consequently, chloroplast differentiation is blocked and disease symptoms develop. Albicidin is also bactericidal at nanomolar concentrations against a range of Gram-positive and Gram-negative bacteria. This potent and novel antibiotic is especially of interest because of its activity against *Escherichia coli*, a species causing nosocomial diseases. Sequencing and annotation of the entire genome of *X. albilineans* recently revealed that *X. albilineans* possesses 12 large genes encoding nonribosomal peptide synthetases (NRPSs) which are located in four gene clusters covering 4 % of the genome (1). One of these NRPS clusters corresponds to the previously identified albicidin biosynthesis gene cluster. The mode of action of this antibiotic was extensively studied but its structure remains unknown. Characterization of albicidin is the main bottleneck which slows development of its therapeutic application. *X. albilineans* is a slow growing bacterium and production yields of albicidin are low, i.e. it turned out to be extremely tedious to obtain sufficient amounts for structure elucidation. To overcome this problem, we successfully considered heterologous overproduction by transferring all albicidin biosynthesis genes into the fast growing bacterium *Xanthomonas axonopodis* pv. *vesicatoria* (2). Production of albicidin in this heterologous system already allowed us to obtain several milligrams of pure compound and promising preliminary results regarding the structural characterization of albicidin. As an example, ¹H-NMR analyses showed the presence of a number of para-substituted aromates, putatively tyrosine or 4-hydroxyphenylglycines. However, the number of amide protons found in ¹H-NMR spectra is lower than the number expected from the biosynthesis assembly lines, suggesting the involvement of tailoring steps during or post-NRPS biosynthesis.

In silico analysis of the three other NRPS gene clusters resulted in partial prediction of the sequences of the precursor peptides synthesized by these clusters which do not resemble any peptide described to date. One of these NRPS gene clusters encodes a complete machinery predicted to be required for secretion and tailoring of small molecules: ABC transporter, MbtH like protein, isomerase, aminotransferase, acyltransferase, enoyl-CoA hydratase. The two other NRPS gene clusters encode only NRPSs which are thought to trans-act with the first one. Interestingly, the NRPSs encoded by *X. albilineans* share characteristics with NRPSs encoded by the root and stem-nodulating *Bradyrhizobium* sp. BTAi1, suggesting structural similarities between small molecules produced by these two plant interacting species. In *X. albilineans*, functional analyses of the phosphopantetheinyl transferase gene (which is required for activation of NRPSs) showed that these non-albicidin NRPS gene clusters are most likely involved in the biosynthesis of at least one new virulence factor.

Future work will focus on characterization of the full structure of albicidin as well as the isolation and characterization of other small molecules assembled by NRPS. These compounds will be chromatographically isolated and characterized by high-resolving FTICR mass spectrometry, 2D-NMR spectroscopy and X-ray crystallography. Isolation and structural characterization of these new bioactive molecules will facilitate the annotation of biosynthesis gene clusters and the study of their role during interactions between sugarcane and *X. albilineans*. On a biochemical level, deciphering their role in pathogenicity should result in the identification of new mechanism(s) involved in xylem colonization by bacterial plant pathogens and give important insights for crop protection.

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